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Effects of cooking and screw-pressing on functional properties of protein in milkweed (*Asclepias* spp.) seed meals and press cakes

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ABSTRACT

This study determined the effects of oil processing conditions on functional properties of milkweed seed proteins to evaluate their potential for value-added uses. Flaked milkweed seeds were cooked at 82 °C (180 °F) for 30, 60 or 90 min in the seed conditioner, and then screw-pressed to extract the oil. Proximate composition and protein functional properties of cooked flakes and press cakes were determined and compared with those of unprocessed ground, defatted milkweed seeds. Milkweed seed protein was most soluble at the pH range of 7–10, had excellent emulsifying properties, and produced substantial but highly unstable foams. Heat applied during seed cooking and screw-pressing did not reduce protein solubility and improved emulsifying, foaming, and water-holding capacities. Emulsifying capacity was much higher at pH 10 than at pH 7. These results showed that the protein in both the milkweed seed and its press cake from oil processing has useful functional properties that could be utilized in applications such as paint emulsifier and adhesive extender.

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1. Introduction

Milkweed (*Asclepias* spp.) is an industrial crop that is grown primarily for its seed pods, which produce floss that provide high thermal insulation (Crews et al., 1991). Natural Fiber Corp. (Ogallala, NE) is the commercial producer of milkweed seed floss as hypoallergenic fiber fillers in comforters and pillows (Knudsen and Zeller, 1993). Milkweed seeds are mere by-products of floss production and have limited uses as plants for landscaping and erosion control.

Whole milkweed seeds are made up of 37% kernel, 51% hulls, and 12% wings (paper-thin extension around the edge of the hull). Seeds contain 21% oil (dry basis, db), nearly 75% of which is found in the kernel and the remainder in the hulls (Evangelista, 2007). Refined milkweed seed oil contained an abundance of unsaturated fatty acids (34% oleic, 50% linoleic, 1% linolenic) and had poor oxidative stability, but the oil could serve as an alternative triglyceride source (Holser, 2003). The meal, however, is not suitable for animal feeds because milkweed contains cardiac glycosides (cardenolides).

Evangelista (2007) also reported that milkweed seeds contain 32% crude protein (db). The kernel has 39% protein, which accounts for 45% of total protein. The hull also contains substantial protein, 31% (db), which represents about half of the total protein based on the distribution of hulls in the whole seed. There is scarce information available about the quality and properties of milkweed seed protein. An earlier research by Gerhardt (1930) found that almost 50% of the total

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nitrogen in milkweed seeds was water-extractable. Of this fraction, proteins precipitated by heat (albumins) made up 51% and peptones and diamino acids accounted for 28%. More recently, we completed a pioneering study (Hojilla-Evangelista et al., 2008) that determined the proximate composition, amino acid profile, molecular weights of fractions, soluble classes, and functional properties of milkweed seed proteins. We detected 12 bands in milkweed seed protein, with the 8 major bands resolving between 6.5 and 96 kDa. We found that the soluble protein classes were distributed as follows: water-soluble (albumins), 22%; salt-soluble (globulins), 15%; ethanol-soluble (prolamins), 12%; and, NaOH-soluble (glutelins), 3.5%. We noted that milkweed seed protein was least soluble (12%) at pH 4, had 40% solubility at pH 7, and most soluble (60%) at pH 10. The protein also had excellent emulsification properties and produced satisfactory foam volumes, but the foams collapsed rapidly. The present study is a continuation of our previous work and was conducted to determine the effects of oil processing conditions on the functional properties of milkweed seed protein and identify possible value-added uses for milkweed seed meal and press cake.

2. Materials and methods

2.1. *Starting materials*

Whole milkweed seeds were provided by Natural Fiber Corp. (Ogallala, NE). Seeds were cleaned by screening and aspiration. Seeds were flaked to 0.25 mm (0.01 in.) thickness by using a Roskamp flaking mill (Model SP900-12, CPM Roskamp Champion, Waterloo, IA). Flaked seeds were heated to 82 °C (180 °F) in the laboratory seed conditioner (Model 324, French Oil Mill Machinery Co., Piqua, OH) to obtain cooked flakes. Residence times in the conditioner were 30 (time needed to reach 82 °C), 60, and 90 min. Cooked flakes were thus designated as CF30, CF60, and CF90, respectively. Their corresponding press cakes (PC) were obtained after oil extraction with a heavy-duty laboratory screw-press (Model L-250, French Oil Mill Machinery Co., Piqua, OH).

2.2. *Proximate analyses*

Unprocessed milkweed seeds, cooked flakes, and press cakes from oil processing were ground into ca. 30-mesh particle size by using a Cuisinart coffee grinder (Model DCG-12BC, East Windsor, NJ) for 2 min. Drying of samples prior to analyses was not necessary. Moisture, crude protein ($\%N \times 6.25$), and crude oil contents of the samples were determined by using AOCS standard methods Ba 2a-38, Ba 4e-93, and Ba 3-38, respectively (AOCS, 1998).

2.3. *Native gel electrophoresis*

Electrophoresis of native proteins was done according to the procedure we reported previously for *Cuphea* (Hojilla-Evangelista and Evangelista, 2006). Ground, unprocessed milkweed seeds, cooked flakes, and press cakes were weighed into centrifuge tubes to provide 4 mg protein/mL in 500 μL of commercial native sample buffer (Invitrogen Tris–glycine native sample buffer, pH 8.6, Invitrogen Corp., Carlsbad, CA). The tubes were shaken for 10 min on a platform shaker and then centrifuged at $5000 \times g$ for 5 min. Twenty μL of supernatants were loaded onto pre-cast Novex^{TM} Tris–glycine 6–18% gradient gel. Unstained protein standards (Invitrogen NativeMark^{TM}, M.W. from 20 to 1236 kDa) were included in the gel. Electrophoresis was done at 125 V for 78 min in an Invitrogen XCell SureLock^{TM} Mini-Cell system. The running buffer was Invitrogen Tris–glycine native running buffer (25 mM Tris base, 192 mM glycine), pH 8.3, which was diluted to 10× volume with nanopure water before use.

2.4. *Functionality tests*

Ground unprocessed milkweed seeds, cooked flakes, and press cakes were prepared for protein functionality testing by following the method that we used for *Cuphea* (Hojilla-Evangelista and Evangelista, 2006), which involved defatting at 25 °C using hexane. We have determined from our previous work that this method provided satisfactory preparation for functional property testing of near-native state protein, as long as the residual oil content of the sample was ≤0.5% (db). Six extraction cycles were done to obtain the desired residual oil content in the meal. In each extraction, the mixture (1 g sample:10 mL solvent) was stirred for 1 h with a magnetic bar. The mixture was allowed to stand until the supernatant has cleared, and then the solvent layer was pipetted out and discarded. Defatted ground samples were air-dried in a fume hood until the hexane smell was no longer detectable, and then stored in screw-capped polyethylene containers at room temperature until use.

2.4.1. *Protein solubility*

Protein solubilities of samples containing 10 mg protein/mL were determined at pH 2.0, 4.0, 5.5, 7.0, 8.5, and 10.0 according to the method of Balmaceda et al. (1984). The amounts of soluble protein in the supernatants were determined spectrophotometrically using the Biuret method. Bovine serum albumin was the protein used to generate the standard curve.

2.4.2. *Surface hydrophobicity*

Surface hydrophobicity indices (S_o) of soluble proteins in the extracts were determined at pH 7.0 and 10.0 as described by Hojilla-Evangelista et al. (2004), which was adapted from the method of Sorgentini et al. (1995). Samples were weighed out to provide 2 mg protein/mL and dispersed in 0.01 M phosphate buffer (pH 7) or 0.025 M $NaHCO_3{\cdot}Na_2CO_3$ buffer (pH 10). Supernatants were diluted with the pH 7 or pH 10 buffer to yield 1/5, 1/10, 1/50, 1/100, and 1/500 concentrations of the starting protein content. The fluorescence probe was 8.0 mM 8-anilino-1-naphthalene sulfonate (ANS). Fluorescence intensities (FIs) were measured by a Varian Cary Eclipse Fluorescence Spectrophotometer (Walnut Creek, CA) at a slit opening of 10 nm and wavelengths of 350 nm (excitation) and 525 nm (emission). FI values were plotted against protein concentrations to determine S_o, which corresponded to the initial slope of the graph as calculated by linear regression.

2.4.3. *Foaming properties*

Foam capacity and stability of samples (10 mg protein/mL) were determined at neutral pH and at the pH where protein solubility was greatest by following exactly the procedure described by Myers et al. (1994). Foam capacity was the volume (mL) of foam produced in 1 min. Foam stability was expressed as the % foam remaining after standing for 15 min.

2.4.4. *Emulsifying properties*

Emulsifying properties were determined according to the method of Wu et al. (1998). Samples were weighed out to provide 1 mg protein/mL and dispersed in 0.01 M phosphate buffer (pH 7) or 0.025 M $NaHCO_3 \cdot Na_2CO_3$ buffer (pH 10). Small amounts of 0.1 M NaOH were added to attain a final sample pH of 7.0 or 10.0. Mixtures were allowed to stand for 15 min. Emulsions were prepared by homogenizing mixtures of 6 mL sample supernatants and 2 mL corn oil with a hand-held homogenizer operated at high setting (20,000 rpm) for 1 min. Emulsification activity index (EAI, in m^2/g) and emulsion stability index (ESI, in min) were calculated from absorbance readings taken at 500 nm.

2.4.5. *Water-holding capacity (WHC)*

WHC of the samples was determined by adapting the method of Balmaceda et al. (1984). We modified the method in the following ways: capped tubes with samples were placed on a platform shaker for 15 min instead of being stirred individually, sample pH was adjusted to 7.0 or 10.0 by adding 0.1 M NaOH, and a water-bath set at 60 °C was used for heating instead of a hot plate. All other steps and calculations were done exactly as described in the original method.

2.5. *Statistical analyses*

Statistical analyses were performed by using the SAS® Systems for Windows software (SAS Institute Inc., Cary, NC). Analysis of variance and Bonferroni *t*-tests or Duncan's Multiple Range tests were performed on duplicate replications of data to determine significant differences among the treatments ($p \leq 0.05$).

3. Results and discussion

3.1. *Moisture, oil and protein contents*

Gerhardt (1930) reported previously that milkweed seeds contained 21.2% oil, 37.5% crude protein, 11.5% crude fiber, and 4.1% ash. We obtained a similar amount of crude oil in our unprocessed milkweed seed (Table 1) and the value was comparable to oil contents reported for cottonseed and soybean (Wolf, 1983). Our protein content of about 30% (dry basis, db) was less than that observed by Gerhardt (1930), but the amount is similar to those of peanut kernel and sunflower seed (Wolf, 1983). The amounts of crude oil and crude protein in our milkweed seed samples were both in the lower range of literature values reported for *A. syriaca* and *A. speciosa*, which were 21–32% for oil and 31–37% for protein (New Crops Database, 1999).

Before defatting with hexane, moisture contents of the flaked seeds decreased when they were held for longer periods in the seed conditioner, as would be expected (Table 1). There was apparently no additional moisture lost during pressing. Heating the flaked seed for 30, 60, or 90 min had no effect on the oil content, while screw-pressing substantially reduced oil contents in the press cakes (Table 1). Crude protein contents of cooked flakes and press cakes remained essentially unchanged under the oil processing conditions employed (Table 1). After defatting with hexane, the oil contents of the ground, unprocessed milkweed, cooked flakes, and press cakes were all ≤0.5% (db). Crude protein values were similar and apparently not affected by hexane-defatting.

3.2. *Electrophoresis results*

The native protein in unprocessed milkweed seed showed three major bands that resolved near 242 kDa, between 480 and 1048 kDa, and 1236 kDa (Fig. 1, lane 2). The dominant subunit, indicated by the darkest color and largest area, resolved between 480 and 1048 kDa. The high MW of the native protein fractions suggested a highly cross-linked structure. The major bands of native proteins in the cooked flakes (lanes 3, 5 and 7) showed patterns similar to the unprocessed seed, but there were additional bands that could be discerned just below the 242 and 146 kDa markers. These bands became more distinct as residence times increased. The CF60 sample also had a faint protein band near the 66 kDa marker (lane 5). This protein sub-unit, as well as those that resolved near 242 and 142 kDa, were more clearly defined and darker-colored in PC60, CF90, and PC90 (lanes 6–8), indicating greater quantities than in the

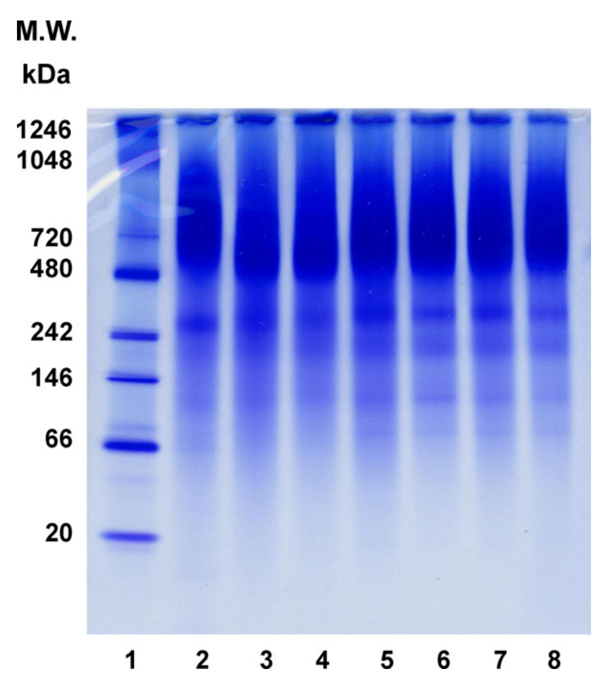

Fig. 1 – Native gel protein band patterns of milkweed seed, cooked flakes (CF), and press cakes (PC): (1) M.W. standards; (2) ground, defatted milkweed seed; (3) CF30; (4) PC30; (5) CF60; (6) PC60; (7) CF90; and (8) PC90. Concentration = 4 mg protein/mL; sample load volume = 20 μL.

Table 1 – Moisture, oil, and protein contents of ground milkweed seeds, cooked flakes, and press cakes.

Sample	Moisture (%)	Crude fat (% db)	Crude protein[a] (% db)	Crude protein (% db, ffb)[b]
Before hexane-defatting				
Milkweed seed	1.8 c	20.8 a	30.4 c	38.4 b
Flakes cooked 30 min (CF30)	7.8 a	20.5 a	37.0 ab	46.6 a
Flakes cooked 60 min (CF60)	4.4 b	19.9 ab	33.5 bc	41.8 ab
Flakes cooked 90 min (CF90)	2.9 bc	22.9 a	31.3 bc	40.6 b
CF30 press cake (PC30)	6.9 a	11.1 c	39.5 a	44.4 a
CF60 press cake (PC60)	4.3 b	12.1 c	36.9 ab	42.0 ab
CF90 press cake (PC90)	3.3 bc	17.7 b	35.3 b	42.9 ab
After hexane-defatting				
Milkweed seed	7.6 a	0.4 a	44.4 a	44.6 a
CF30	10.7 a	0.5 a	47.2 a	47.4 a
CF60	10.0 a	0.4 a	42.6 a	42.8 a
CF90	9.8 a	0.4 a	42.5 a	42.7 a
PC30	8.4 a	0.4 a	47.7 a	47.9 a
PC60	10.2 a	0.2 a	44.2 a	44.3 a
PC90	10.6 a	0.3 a	42.7 a	42.9 a

Values are means of duplicate determinations. In a sample block (before or after defatting), means within a column followed by different letters are significantly different ($p < 0.05$).

[a] Dumas $N \times 6.25$.

[b] db, dry basis; ffb, fat-free basis.

other cooked flakes or unprocessed seed. The presence of the extra bands in the cooked flakes and press cakes implied that heating caused larger protein sub-units to be broken up into smaller units; but, this effect did not appear to be significant or extensive. Thermal treatments of proteins often result in structural changes and hydrolysis of peptide bonds, which, in turn, greatly influence protein functionality (Cheftel et al., 1985).

3.3. *Protein solubility*

Unprocessed milkweed seed protein showed its lowest solubility (14%) at pH 4, had 40% solubility at pH 7, and its highest solubility (60%) at pH 10 (Fig. 2). The presence of alkali typically causes dissociation and disaggregation of proteins, which often lead to improved protein solubility; however, it is also possible that high solubility at alkaline pH could be caused by extensive proteolysis (Kinsella, 1976).

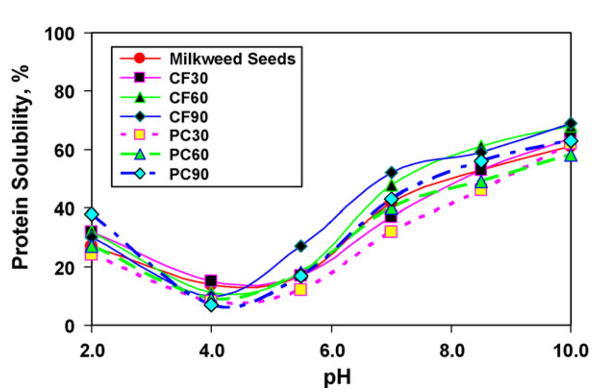

Fig. 2 – Protein solubility profiles of ground, defatted milkweed seed, cooked flakes (CF) heated for 30, 60, and 90 min in the seed conditioner, and corresponding press cakes (PC) from oil processing.

Solubility profiles of proteins from cooked flakes (Fig. 2, solid lines) showed a sigmoidal shape that is similar to that of the unprocessed seed, with the lowest solubilities observed at pH 4 and greatest at pH 10. Heating the flaked seeds for 30 min at 82 °C (CF30) had no apparent effect on protein solubility, as indicated by the nearly identical profiles of CF30 and unprocessed milkweed seed. Cooking for 60 min (CF60) or 90 min (CF90) at 82 °C resulted in improved protein solubilities at pH values of 7 or higher (Fig. 2). The most common response of proteins to heating is irreversible reduction in solubility, because of aggregation of the unfolded protein molecule (Kinsella, 1976; Cheftel et al., 1985). In some instances, however, heating may improve solubility, as long as unfolding of the protein's compact structure is not accompanied by aggregation, because the now-exposed polar side chains can interact with water or another solvent (Cheftel et al., 1985).

The solubility profiles of the press cakes (Fig. 2, broken lines) were also very similar to that of the unprocessed milkweed seed, which indicated that additional heating during screw-pressing had no effect on solubility of proteins, thus allowing for their extraction from the press cake. These results demonstrated that heat applied during cooking and screw-pressing had no deleterious effects on milkweed protein solubility. Protein from unprocessed milkweed seed, cooked flakes, and press cakes showed notable solubilities at pH 7 and pH 10, which indicated potential applications in aqueous systems and high-alkaline environments, such as plywood glues. Thus, the other functional properties were evaluated at both neutral pH and pH 10.

Solubility properties are useful in determining the conditions for extracting and purifying proteins from natural sources, and for fractionating the protein sub-units (Cheftel et al., 1985). Solubility behavior also serves as a good index of the potential applications of the protein, because insolubility is often a measure of denaturation and denatured or aggregated proteins have frequently shown impaired abilities to gel, emulsify or foam effectively (Cheftel et al., 1985).

Table 2 – Functional properties at pH 7 of ground milkweed seeds, cooked flakes (CF) and press cakes (PC).

Sample	Functional properties[a]					
	S_o	EAI (m^2/g)	ESI (min)	FC(mL)	FS (% foam left)	WHC (g water/g protein)
Milkweed seed	165 ± 5 d	102.7 ± 3.8 d	15.4 ± 0.3 ab	101 ± 9 c	39.8 ± 4.0 a	3.70 ± 0.02 b
CF30	167 ± 5 d	102.0 ± 7.4 d	15.9 ± 0.4 ab	105 ± 4 c	0.0 ± 0.0 b	3.06 ± 0.05 b
CF60	177 ± 11 c	98.1 ± 2.1 de	14.2 ± 0.1 b	130 ± 3 a	0.0 ± 0.0 b	2.79 ± 0.02 c
CF90	190 ± 14 b	115.3 ± 15.4 c	18.2 ± 1.1 a	126 ± 14 a	0.0 ± 0.0 b	2.73 ± 0.08 c
PC30	185 ± 0 b	155.0 ± 5.5 a	20.4 ± 0.4 a	116 ± 14 b	0.0 ± 0.0 b	4.66 ± 0.26 a
PC60	209 ± 6 a	134.5 ± 4.7 b	18.8 ± 0.7 a	110 ± 8 bc	0.0 ± 0.0 b	4.80 ± 0.04 a
PC90	204 ± 7 a	96.4 ± 5.2 e	16.3 ± 0.5 ab	104 ± 4 c	37.8 ± 1.1 a	3.51 ± 0.04 b

Values are means ± standard deviations of duplicate determinations. Means within a column followed by different letters are significantly different ($p < 0.05$).

[a] S_o, surface hydrophobicity index; EAI, emulsion activity index; ESI, emulsion stability index; FC, foaming capacity; FS, foam stability; WHC, water-holding capacity.

3.4. *Surface hydrophobicity index (S_o)*

S_o indicates the extent of exposure of hydrophobic regions of protein molecules; higher values suggest more unaggregated proteins. The S_o values for unprocessed milkweed seed protein at pH 7 and pH 10 (Table 2) were significantly lower than the 530 we reported for soybean protein (Hojilla-Evangelista et al., 2004). The markedly lower S_o values implied a substantial presence of aggregated proteins in milkweed seed proteins, which may explain the high MW we observed from native gel electrophoresis (Fig. 1).

At neutral pH, the S_o for milkweed CF increased with prolonged residence times in the seed conditioner (Table 2), which indicated that milkweed seed proteins exposed to the longer cooking period were more unaggregated. PC proteins were even less aggregated and better dispersed, as suggested by their markedly greater S_o values (Table 2). These results support our results from solubility determination (Fig. 2) and provide further evidence of beneficial effects by heat treatment on milkweed seed proteins.

At pH 10, S_o values for protein in unprocessed milkweed seed, CF, and PC (Table 3) were all significantly lower than those obtained at pH 7 (Table 2), although they showed the same trend of greater S_o values with prolonged cooking times. These markedly lower S_o values at pH 10 implied a greater presence of aggregated proteins or degree of denaturation in the cooked flakes and press cakes.

3.5. *Emulsifying properties*

An indicator of a protein's emulsifying capacity is the EAI, which measures the area of oil–water interface stabilized by a unit weight of protein (Wu et al., 1998). Higher EAI values generally indicate better emulsifying capacity. At neutral pH, protein from unprocessed milkweed seed had excellent emulsifying capacity, as shown by an EAI value (Table 2) that was double that of soybean protein (56 m^2/g protein) (Hojilla-Evangelista et al., 2004). Cooking the flakes for up to 60 min did not diminish the protein's emulsifying capacity and their corresponding press cakes (PC 30 and PC60) produced even better emulsifying activities (Table 2). Cooking for 90 min also resulted in higher EAI value.

At pH 10, all the milkweed protein samples had EAI values (Table 3) that were far superior to those observed at pH 7 (Table 2). Heating and limited hydrolysis can bring about improved emulsifying capacity if the protein structure can unfold without resulting in aggregation (Cheftel et al., 1985). The significant increase in EAI for the proteins prepared at pH 10 may have been due to higher quantities of soluble proteins generated by hydrolysis under alkaline conditions.

Table 3 – Functional properties at pH 10 of ground milkweed seeds, cooked flakes (CF) and press cakes (PC).

Sample	Functional properties[a]					
	S_o	EAI (m^2/g)	ESI (min)	FC (mL)	FS (% foam left)	WH9C (g water/g protein)
Milkweed seed	128 ± 2 a	248.8 ± 11.9 a	14.3 ± 0.1 a	116 ± 6 a	4.4 ± 1.5 b	2.91 ± 0.13 bc
CF30	67 ± 1 c	144.8 ± 0.6 c	15.4 ± 0.1 a	108 ± 9 b	3.0 ± 2.2 b	2.90 ± 0.03 bc
CF60	105 ± 6 b	136.9 ± 4.3 d	14.7 ± 0.2 a	110 ± 2 b	3.7 ± 2.7 b	2.80 ± 0.18 c
CF90	108 ± 9 b	134.3 ± 6.3 d	14.6 ± 0.2 a	96 ± 5 c	56.1 ± 7.3 a	2.72 ± 0.07 c
PC30	64 ± 0 c	235.5 ± 4.4 b	16.6 ± 0.5 a	58 ± 1 d	0.0 ± 0.0 b	3.97 ± 0.06 ab
PC60	105 ± 2 b	238.5 ± 1.7 b	14.8 ± 0.5 a	106 ± 0 b	0.0 ± 0.0 b	4.55 ± 0.06 a
PC90	107 ± 3 b	230.6 ± 14.5 b	14.2 ± 0.1 a	105 ± 4 b	0.0 ± 0.0 b	3.59 ± 0.09 bc

Values are means ± standard deviations of duplicate determinations. Means within a column followed by different letters are significantly different ($p < 0.05$).

[a] S_o, surface hydrophobicity index; EAI, emulsion activity index; ESI, emulsion stability index; FC, foaming capacity; FS, foam stability; WHC, water-holding capacity.

ESI values at pH 7 (Table 2) and pH 10 (Table 3) for unprocessed milkweed seed, CF, and PC did not vary from each other, which suggested that the stability of the emulsion formed by the protein was not affected by shifting from neutral to alkaline pH, nor by the heat applied during cooking and screw-pressing. ESI values for milkweed protein from unprocessed seed, CF, and PC were comparable to the 15.0 min reported for soybean protein (Hojilla-Evangelista et al., 2004).

3.6. *Foaming properties*

Unprocessed milkweed seed protein produced ca. 100 mL of foam at pH 7 (Table 2) and cooking the flaked seed for up to 90 min improved the protein's foaming capacities to levels that almost matched the 135 mL of foam generated by the same concentration of soybean protein at neutral pH (Hojilla-Evangelista et al., 2004). Heat-induced unfolding of the protein, provided it is not accompanied by aggregation and loss of solubility, results in improved protein orientation at the interface and greater foam capacity (Cheftel et al., 1985). Screw-pressing did not impart a similar favorable effect, as shown by press cakes having foaming capacities that did not differ greatly from that of unprocessed milkweed seed (Table 2). At pH 10, milkweed protein in the cooked flakes and press cakes had foaming capacities that were less than that of unprocessed seed protein (Table 3). The reduction was particularly severe with the press cake from flakes cooked for 30 min (PC30).

Foams produced by milkweed seed protein were very unstable. At pH 7, only 40% of the foam initially generated remained after 15 min of standing (Table 2) and at pH 10, even far less foam volume remained after the allotted time (Table 3). The heated milkweed seed protein samples generally produced foams that collapsed immediately (Tables 2 and 3). Foam instability arises when there are limited protein–protein interactions at the interface, resulting in weak films that are unable to prevent the leakage of lamellar fluid (Phillips et al., 1994). Milkweed seed may have limited availability of proteins that can participate in interfacial interactions, thus resulting in less-stable foams.

3.7. *Water-holding capacity (WHC)*

WHC determines a protein's ability to impart such properties as texture, body, viscosity, and adhesiveness (Cheftel et al., 1985). At neutral pH, WHC for unprocessed milkweed seed protein (Table 2) was ca. one-half that of the 6.7 g water/g protein reported for soybean protein at neutral pH (Hojilla-Evangelista, unpublished data). Cooking the flaked seeds reduced WHC values slightly, while screw-pressing conditions apparently improved WHC as shown by higher values of the press cakes (Table 2). When WHC was determined under alkaline pH, the values obtained did not vary much from those observed at pH 7. WHC for the cooked flakes were similar to that of the unprocessed seed (Table 3), which suggested that heating did not affect WHC. WHC for the press cake proteins were greater than those of the unprocessed seed and cooked flakes. Heating typically lessens protein WHC, because the denaturation and/or aggregation reduce the availability of polar amino groups for hydrogen bonding with water molecules (Cheftel et al., 1985). But, heating can also unfold the protein and expose side chains that can bind water, resulting in improved WHC. This may explain the higher WHC values for the press cake proteins.

4. Conclusions

Milkweed seed protein was most soluble at pH ≥ 7.0, had excellent emulsifying properties, and produced substantial but highly unstable foams. Heat treatment during seed cooking and screw-pressing had no detrimental effect on protein solubility and improved emulsifying capacity, foaming capacity, and water-holding capacity of the protein. Emulsifying capacity was the only functional property that was affected significantly by pH, with values for both unprocessed and heated samples markedly greater at pH 10 than at pH 7. These results showed that the press cake from oil processing of milkweed seed contains protein that still has useful functional properties, and, like milkweed seed, could be utilized as paint emulsifier, adhesive extender, and thickener.

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